AWARD NUMBER: W81XWH-12-1-0629

TITLE: Novel Genetic Models to Study the Role of Inflammation in Brain Injury-Induced Alzheimer's Pathology

PRINCIPAL INVESTIGATOR: Bruce T. Lamb

CONTRACTING ORGANIZATION: The Cleveland Clinic Foundation
Cleveland, OH 44195

REPORT DATE: December 2015

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2015	**Final** Addendum	30Sep2014 - 29Sep2015

4. TITLE AND SUBTITLE

Novel Genetic Models to Study the Role of Inflammation in Brain Injury-Induced Alzheimer's Pathology

5a. CONTRACT NUMBER
12109018 TATRC

5b. GRANT NUMBER
W81XWH-12-1-0629

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bruce T. Lamb; Olga Kokiko-Cochran

E-Mail: lambb@ccf.org; kokikoo@ccf.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Cleveland Clinic Foundation
9500 Euclid Avenue
Cleveland, OH 44195

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Individuals exposed to traumatic brain injury (TBI) are at a greatly increased risk for developing a number of neurodegenerative diseases including Alzheimer's disease (AD). TBI promotes the development of the pathological hallmarks of AD including production and extracellular deposition of the beta -amyloid peptide in senile plaques and intracellular aggregation of hyperphosphorylated, microtubule-associated protein tau (MAPT) in neurofibrillary tangles (NFTs). Several lines of evidence suggest that altered monocyte infiltration and microglial activation may be directly involved in the pathogenesis of both beta-amyloid and MAPT pathologies. The primary hypothesis to be tested in the current studies is that TBI induces infiltration of peripheral monocytes as well as acute and local activation of brain microglia within the injured brain and that these two cell types play roles distinct from each other in inducing both beta-amyloid pathologies and MAPT phosphorylation and aggregation leading to chronic pathological conditions that pre-dispose individuals exposed to TBI to develop AD later in life. Here we report that TBI results in a widespread neuroinflammatory response including microglial activation and monocyte infiltration. Interestingly, the acute macrophage response to TBI is reduced in a mouse model of amyloid pathology (R1.40) compared to control mice; however, long-term behavioral outcome and neurodegeneration are worse at chronic post-injury time points. By contrast, the acute macrophage response to TBI in hTau mice is enhanced compared to brain injured controls. Brain injured mice display region specific macrophage activation at chronic time points independent of behavioral impairment. Subsequent studies revealed the presence of infiltrating monocytes near the injury cavity at acute time points and in sub-cortical structures at chronic time points in all brain injured mice. Ongoing studies will confirm the unique expression profile of monocytes and microglia at acute and chronic time points. Taken together, these novel results suggest that TBI impacts amyloid and MAPT pathologies quite differently and have implications for immunomodulatory therapies for TBI.

15. SUBJECT TERMS
Alzheimer's, brain injury, beta-amyloid, MAPT, inflammation, monocytes, microglia

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. Introduction

Exposure to traumatic brain injury (TBI) has numerous acute and chronic sequelae, including an increased risk for the development of Alzheimer's disease (AD) [1-3]. Mechanisms contributing to these pathological events are not well characterized; however, they are often associated with neuroinflammation. Postinjury neuroinflammation is characterized by activation of brain-resident microglia as well as infiltration and activation of monocytes due to disruption of the blood-brain barrier [4-6]. Assessing the exact roles of these cells in AD pathogenesis has proven exceedingly difficult as no immunohistochemical markers unambiguously differentiate monocytes from microglia, particularly in the activated (macrophage) state. Although there is increasing experimental evidence that macrophages derived from recruited monocytes can regulate Aβ pathologies, the effects of monocytes on other AD phenotypes, or the role of these cells in TBI induced AD pathologies have yet to be established. The central hypothesis of the current studies is that monocytes and microglia play roles decisively distinct from each other in the development of AD-like pathologies following TBI. Furthermore, these functions are exerted differentially in the varied pathological processes that underlie AD, including generation and extracellular deposition of beta-amyloid (Aβ) and phosphorylation and intracellular aggregation of microtubule associated protein tau (MAPT).

2. Keywords

Traumatic brain injury; Alzheimer's disease; beta-amyloid; microtubule associated protein tau; macrophage; microglia; monocyte; fluid percussion injury; mouse

3. Accomplishments

Major Goals of the Project

Approvals and Training of Staff

Task 1. Complete required approval documents for the studies; Completed Quarter 30-09-2012 to 31-12-2012
Task 2. Provide required training for staff; Completed Quarter 30-09-2012 to 31-12-2012

Specific Aim 1

Task 1. Generate animals required for studies; Completed Quarter 30-09-2012 to 31-12-2012
Task 2. Perform FPI; Completed Quarter 01-01-2013 to 31-03-2013
Task 3. Perform behavioral analysis; Completed Quarter 01-04-2013 to 30-06-2013
Task 4. Analysis of brain tissue; Completed Quarter 01-04-2013 to 30-06-2013
Task 5. Published manuscript on results from Specific Aim 1; Completed Quarter 01-07-2015 to 30-09-2015

Specific Aim 2

Task 1. Generate animals required for studies; Completed Quarter 01-01-2013 to 31-03-2013
Task 2. Perform FPI; Completed Quarter 01-01-2014 to 31-03-2014
Task 3. Perform behavioral analysis; Completed Quarter 01-10-14 to 31-12-14
Task 4. Analysis of brain tissue; Completed Quarter 01-04-2015 to 30-06-2015
Task 5. Published manuscript on results from Specific Aim 2; Final data is currently being organized into a manuscript

Specific Aim 3

Task 1. Generate animals required for studies; Completed Quarter 01-07-13 to 30-09-2013
Task 2. Perform FPI; Completed Quarter 01-01-2014 to 31-03-2014
Task 3. Analysis of brain tissue; Completed Quarter 01-07-2015 to 30-09-2015
Task 4. Purification of monocytes/microglia; Completed Quarter 01-07-2015 to 30-09-2015
Task 5. Gene expression microarray analysis; RNA has been isolated for gene expression analysis
Task 6. Multi-photon microscopy; Completed Quarter 01-07-2015 to 30-09-2015
Task 7. Published manuscript on results from Specific Aim 1; Following gene expression analysis of sorted cells, a manuscript will be prepared

Accomplished Under These Goals

Specific Aim 1

Results from Specific Aim 1 were published in Journal of Neurotrauma in October 2015 (see Appendix).

Specific Aim 2

As previously discussed in recent technical progress reports, the studies in Specific Aim 2 have revealed an enhanced macrophage response to TBI at acute time points in hTau mice compared to control Non-Tg mice. The macrophage response substantially decline by 120 DPI and no significant differences were detected between TBI and sham mice regardless of genotype. Furthermore, TBI did not result in chronic behavioral disturbances or worsened tau pathology in hTau mice. These results were contrary to what was predicted following pilot studies using the Dragonfly FPI device. The milder and more diffuse injury produced by the Amscien Instruments FPI device revealed an important effect of injury severity that should be considered in future studies. Together, these data highlight the potentially unique role of injury severity in altering the macrophage response. Fewer infiltrating cells were identified following TBI with the Amscien FPI device compared to the Dragonfly FPI device. This observation suggests that infiltrating cells may promote tau-related pathology; however, additional experiments and a more thorough characterization of these effects must be completed before confirmation. It should be noted that experiments further characterizing tau phosphorylation and aggregation are ongoing. Recent quantification results are included below.

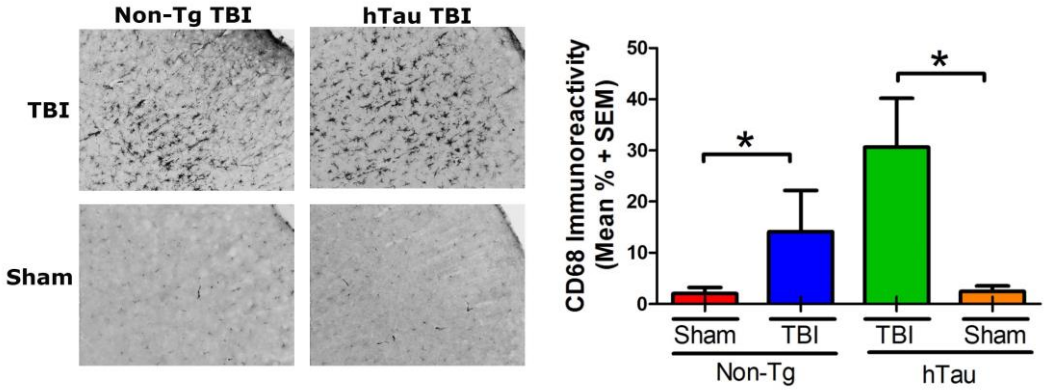

Figure 1. CD68 Immunoreactivity is Enhanced in hTau mice at 3 DPI. CD68+ cells were identified in close proximity to the injury cavity in TBI mice with hTau TBI mice showing widespread CD68 immunoreactivity in the cortex. **B**) Quantification of the percent area covered by CD68 immunoreactivity, which was enhanced in hTau TBI mice compared to Non-Tg TBI mice.

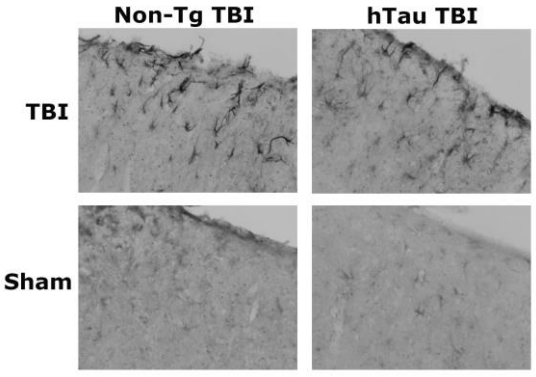

Figure 2. Trem2 Immunoreactivity at 3 DPI. Trem2 immunoreactivity was apparent near the injury site in all mice, including TBI and sham mice. Trem2 immunoreactivity was enhanced in TBI mice compared to shams; however, variability between animals prevents any statistically significant differences from being observed.

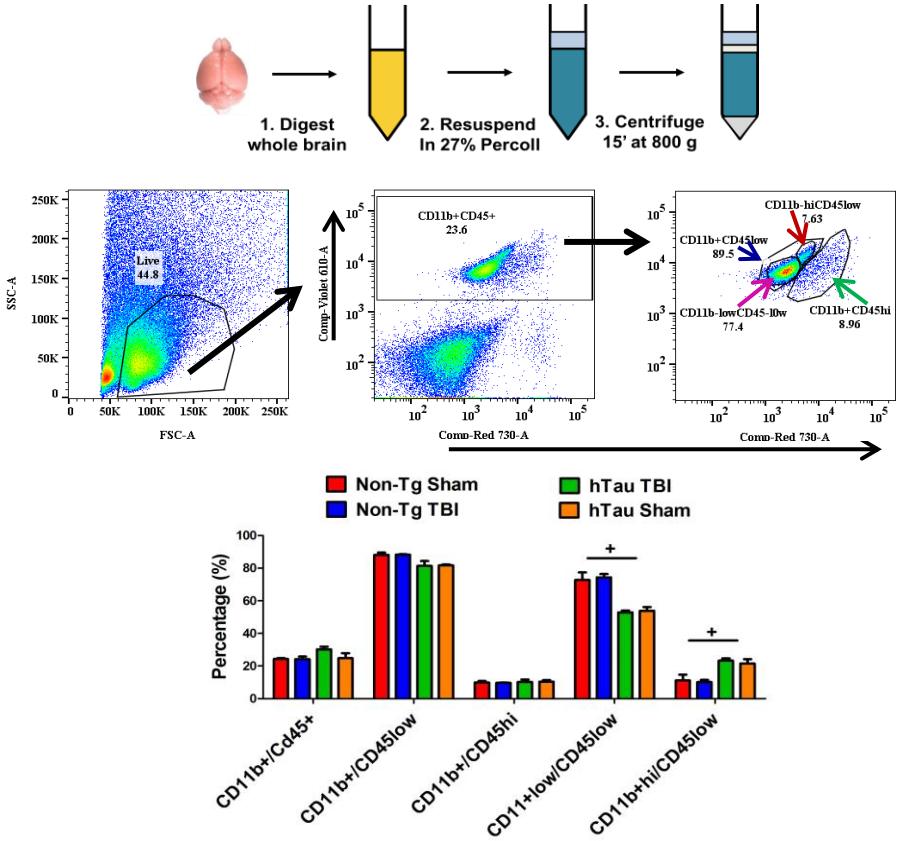

Figure 3. Activated Microglia Persist at 120 DPI in hTau Mice. Mononuclear phagocytes were isolated at 120 DPI via Percoll gradients from TBI and sham injured Non-Tg and hTau mice. Isolated cells were stained with antibodies against Cd11b and CD45. Distinct cell populations identified were: 1) macrophages (CD11b+/CD45+), 2) microglia (CD11b+/CD45low), 3) monocytes (CD11b+/CD45hi), 4) "resting" microglia (CD11b+low/CD45low), and 5) "activated" microglia (CD11b+hi/CD45low). Notably, a higher proportion of activated microglia was identified in the brains of htau mice compared to Non-Tg mice regardless of injury group at 120 DPI.

Specific Aim 3

Experiments in Specific Aim 3 were substantially delayed due to problems with breeding hTau mice as well as unforeseen setbacks in optimizing an isolation protocol for flow cytometry experiments. Since the last progress report, substantial effort has been spent isolating RNA from sorted cell populations. We plan to move forward with sequencing analysis of microglia and monocytes from TBI and sham mice at 3 and 120 DPI in all genotypes (Non-Tg, R1.40, and hTau).

Opportunities for Training and Professional Development

Dr. Kokiko-Cochran attended the annual National Neurotrauma Symposium (NNS) in 2013 and 2015 as well as the Society for Neuroscience (SFN) conference from 2012-2015. Dr. Kokiko-Cochran also attended various local research events including the Neurological Institute Research Day (NIRD) at the Cleveland Clinic as well as Concussion 2015, sponsored by the National Academy of Engineering, the Institute of Medicine, and Case Western Reserve University. Dr. Kokiko-Cochran presented posters at many of these events and gave a nanosymposium at the SFN meeting in 2014. Attending these events provided Dr. Kokiko-Cochran with an opportunity to network with colleagues from around the world and to discuss the data generated under this award. Ultimately, these experiences served as a platform to disseminate results and to discuss the potentially unique roles of microglia and monocytes in mediating post-injury beta-amyloid and tau pathology following TBI.

The Department of Neurosciences hosts a weekly Fellow's Seminar series in which all graduate students and post-doctoral fellows present their data. Dr. Kokiko-Cochran presented data generated in this grant in 2013-2015. The Lamb laboratory also maintains a regular journal club in which Dr. Kokiko-Cochran presented articles relevant to the topic of macrophage mediated neuroinflammation. See Products section for detailed list of conference presentation and seminars.

Dissemination of Results to Communities of Interest
Nothing to Report

Future Plans for Next Reporting Period
Nothing to Report

4. Impact

Impact of the Development of the Principal Discipline of the Project
One goal of this project was to identify the distinct contribution of brain-resident microglia and infiltrating peripheral monocytes in recovery following brain injury at an acute and chronic post-injury time point. The use of novel mouse models with targeted use of specific chemokines facilitated identification of the spatiotemporal distribution of microglia and monocytes following brain injury as well as the potentially unique gene expression profile of microglia and monocytes in the context of accumulating beta-amyloid or pathological human tau. The neurotrauma field has only recently begun to appreciate the truly significant role of the innate immune response in recovery following brain injury. Studies characterizing the role of peripheral immune cells as well as the interaction between peripheral and central immune cells following TBI have been lacking until recently. Thus the results of these studies are very timely and add significant support to the idea that the peripheral immune response is a key mediator in outcome following brain injury and persistent involvement of the peripheral immune response represents a unique target for therapeutic intervention.

Impact on Other Diciplines
This project focused on establishing the role of brain-injury induced neuroinflammation in promoting AD-like phenotypes in various mouse models. Specifically, we sought to characterize the unique role of brain resident microglia and infiltrating peripheral monocytes in recovery following TBI. Thus, there is great translational potential in the results. First, we have focused on characterizing the macrophage response following a single, moderate TBI. A thorough characterization of the macrophage response following other types of TBI has not been completed. Future studies could examine the role of microglia and monocytes in the context of blast TBI as well as repetitive TBI, which has been associated with development of chronic traumatic encephalopathy (CTE). Second, accumulating evidence suggests that infiltrating monocytes are actively involved in mediating recovery following ischemia [7], another avenue in which brain injury can occur and often a consequence of TBI. Comparing results from TBI and stroke studies will be important is thoroughly characterizing the macrophage response and the potentially beneficial or detrimental effects of microglia and monocyte activation. Third, AD is one of many neurodegenerative diseases that have been correlated with incidence of TBI. It is possible that the macrophage response, if not resolved following TBI, promotes development of other neurodegenerative diseases as well. This may certainly depend on the location and severity of injury, but could be further explored in subsequent experimental studies.

Impact on Technology Transfer
Nothing significant to report.

Impact on Society Beyond Science and Technology
The results from this study lend support to accumulating evidence linking TBI to neurodegenerative disease. In these experiments, TBI induced several key pathological features of AD including activation of brain resident microglia, infiltrating of peripheral monocytes, and increased production and release of inflammatory cytokines which persisted at chronic post-injury time points. In addition, brain injury enhanced production of APP and promoted tau phosphorylation. Certainly the general population may not appreciate identification of specific molecular pathways linking TBI to AD; however, these results support the notion that persistent neuroinflammation is detrimental. Furthermore, these results showcase the chronic consequences of TBI and support initiatives to educate the general population on ways to prevent brain injury as well as the long-term consequences of brain injury.

5. Changes/Problems

Three significant problems occurred during the completion of this project which delayed progress. First, we began these experiments with a fluid percussion injury (FPI) device manufactured by Dragonfly Research & Development, Inc. Unfortunately, the device from Dragonfly malfunctioned and was not repairable following completion of studies for Specific Aim 1. As a result we transitioned to using a FPI device manufactured by Amscien Instruments. There was a distinct difference in the brain injury produced by each device. Most obviously, there was a focal lesion cavity produced by the Dragonfly device and a diffuse contusion produced by the Amscien device. Experiments that had been initiated for Specific Aims 2 and 3 had to be repeated. Although the TBI produced by the Amscien device is characteristic of a moderate FPI, the change in device made it challenging to compare the results of Specific Aim 1 to those of Specific Aims 2 and 3 because the potential differences in injury severity. Second, we experienced delays in generating and subsequently breeding hTau/ $CX3CR1^{GFP/+}$/$CCR2^{RFP/+}$ mice which postponed completion of Specific Aim 3. Third, we spent a significant amount of time optimizing the macrophage isolation protocol for flow cytometry studies aiming to sort microglia and monocytes in $CX3CR1^{GFP/+}$/$CCR2^{RFP/+}$, R1.40/ $CX3CR1^{GFP/+}$/$CCR2^{RFP/+}$, and hTau/ $CX3CR1^{GFP/+}$/$CCR2^{RFP/+}$ mice. As a result, we were unable to complete the RNA sequencing studies before the grant ended. It should be noted that we now have RNA from isolated GFP+ and RFP+ sorted cell populations in Non-Tg, R1.40 and hTau mice at 3 and 120 DPI following sham or brain injury and sequencing studies will be completed.

6. Products

Abstracts

- Kokiko-Cochran, O., Veenstra, M., Ransohoff, L., Bhaskar, K., Lee, Y-S, Lamb, B. "Traumatic brain injury distinctly influences amyloid and tau pathology", Poster presented at the annual SFN meeting, New Orleans, LA, October, 2012.
- Kokiko-Cochran, O., Ransohoff, L., Bhaskar, K., Lee, Y-S, Lamb, B. "Traumatic brain injury induces a distinct inflammatory response in a genomic based model of Alzheimer's disease", Poster presented at the annual NNS meeting, Nashville, TN, August, 2013.
- Kokiko-Cochran, O.N., Ransohoff, L., Lee, S., Lee, Y-S, Lamb, B.T. Traumatic Brain Injury Induces a Reduced Inflammatory Response at Early Timepoints, but Worsens Outcomes at Late Timepoints in a Mouse Model of Alzheimer's Disease. Poster presented at the annual SFN meeting, San Diego, California, 2013
- Kokiko-Cochran, O.N., Ransohoff, L., Veenstra, M., Lee, S., Sikora, M., Teknipp, R., Xu, G., Bemiller, S., Wilson, G., Crish, S., Bhaskar, K., Lee, Y-S., Ransohoff, R., Lamb, B.T. Accumulating beta-amyloid alters the post-injury inflammatory response. Poster presented at the annual NNS meeting, Santa Fe, New Mexico, 2015.
- Kokiko-Cochran, O.N., Saber, M., Teknipp, R., Bemiller, S., Lamb, B.T. Wild-type human tau enhances the macrophage response to experimental traumatic brain injury. Poster presented at the annual SFN meeting, Chicago, IL, 2015.

Seminars

- *Annual Neuroscience Fellow's Seminar Series, Lerner Research Institute, Cleveland Clinic.* (2013) "Opposing Acute and Chronic Effects of Traumatic Brain Injury in a Mouse Model of Alzheimer's Disease" Kokiko-Cochran, O.N.
- *Annual Neuroscience Fellow's Seminar Series, Lerner Research Institute, Cleveland Clinic.* (2014) "Altered neuroinflammation, neurodegeneration, and behavior following traumatic brain injury in a mouse model of Alzheimer's disease" Kokiko-Cochran, O.N.
- *44th Annual Society for Neuroscience meeting; nanosymposium, Washington, D.C.* (2014) "Traumatic brain injury induces a distinct macrophage response at acute and chronic time points in a mouse model of Alzheimer's disease" Kokiko-Cochran, O.N., Saber, M., Teknipp, R., Ransohoff, R.M., Lamb, B.T.

Publications

- Kokiko-Cochran, O.N., Ransohoff, L., Veenstra, M., Lee, S., Sikora, M., Teknipp, R., Saber, M. Xu, G., Bemiller, S., Wilson, G., Crish, S., Bhaskar, K., Lee, Y-S., Ransohoff, R.M., & Lamb, B.T. (2015). Altered neuroinflammation, neurodegeneration and behavior following traumatic brain injury in a mouse model of Alzheimer's disease. *(J Neurotrauma*, Epub ahead of print*)*.

Animal Models

- CX3CR1$^{GFP/+}$/CCR2$^{RFP/+}$, R1.40/ CX3CR1$^{GFP/+}$/CCR2$^{RFP/+}$, and hTau/ CX3CR1$^{GFP/+}$/CCR2$^{RFP/+}$ mice generated and available to requesting investigators. All models are also deposited at The Jackson Laboratory.

Grant Funding

- NINDS R21, NS087298, PIs: B.T. Lamb and R.M. Ransohoff, "The Role of Monocytes and Microglia in Traumatic Brain Injury-Induced Tauopathies, 7/1/13-6/30/15.
- TATRC MRPRA, W81XWH-14-1-0264, ERMS#13321017, PIs: L. Goldstein and B.T. Lamb, "Effects of Blast Neurotrauma on Alzheimer's Disease Pathogenesis", 10/1/13-9/31/15.
- CDMRP, W81XWH-15-1-0257, AZ140162, PIs: B.T. Lamb, C. Bernick, "The role of inflammation in development of Alzheimer's disease following repetitive head trauma," 10/1/15-9/31/2018.

7. Participants & Other Collaborating Organizations

Name:	Bruce T. Lamb
Project Role:	Primary Investigator
Nearest person month worked:	2.6
Contribution to Project:	Dr. Lamb managed all personnel involved in this project and provided expertise in experimental design, data analysis and interpretation of results, as well as manuscript preparation.

Name:	Olga N. Kokiko-Cochran
Project Role:	Post-doc
Nearest person month worked:	23.8
Contribution to Project:	Dr. Kokiko-Cochran performed all experiments in this grant with technical support in behavioral testing, tissue processing, and statistical analyses.

Name:	Ryan Teknipp
Project Role:	Technician
Nearest person month worked:	19.4
Contribution to Project:	Mr. Teknipp maintained the breeding colony for this project and assisted with behavioral testing and tissue processing.

Name:	Matt Sikora
Project Role:	Technician
Nearest person month worked:	9.2
Contribution to Project:	Mr. Sikora maintained the breeding colony for this project and assisted with behavioral testing and tissue processing.

Name:	Anna Rietsch
Project Role:	Technician
Nearest person month worked:	5.7
Contribution to Project:	Ms. Rietsch assisted with optimization and completion of flow cytometry studies.

Name:	Valerie Swank
Project Role:	Technician
Nearest person month worked:	2.2
Contribution to Project:	Ms. Swank maintained the breeding colony for this project and assisted with tissue processing.

Name:	Daniel Kim
Project Role:	Research Assistant
Nearest person month worked:	1.2
Contribution to Project:	Mr. Kim assisted with optimization of flow cytometry studies.

Change in Active other Support of the PI
Nothing to Report

Other Organizations Involved as Partners
Nothing to Report

8. Special Reporting Requirements
Nothing to Report

9. Appendices

References

1. Fujimoto, S.T., et al., *Motor and cognitive function evaluation following experimental traumatic brain injury.* Neurosci Biobehav Rev, 2004. **28**(4): p. 365-78.
2. Tokuda, T., et al., *Re-examination of ex-boxers' brains using immunohistochemistry with antibodies to amyloid beta-protein and tau protein.* Acta Neuropathol, 1991. **82**(4): p. 280-5.
3. Guo, Z., et al., *Head injury and the risk of AD in the MIRAGE study.* Neurology, 2000. **54**(6): p. 1316-23.
4. Pierce, J.E., et al., *Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats.* Neuroscience, 1998. **87**(2): p. 359-69.
5. Smith, D.H., et al., *Progressive atrophy and neuron death for one year following brain trauma in the rat.* J Neurotrauma, 1997. **14**(10): p. 715-27.
6. Soares, H.D., et al., *Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury.* J Neurosci, 1995. **15**(12): p. 8223-33.
7. Gliem, M., M. Schwaninger, and S. Jander, *Protective features of peripheral monocytes/macrophages in stroke.* Biochim Biophys Acta, 2015.